Distribution of Lymphocytes With Interleukin-2 Receptors (TAC Antigens) in Reactive Lymphoproliferative Processes, Hodgkin's Disease, and Non-Hodgkin's Lymphomas

An Immunohistologic Study of 300 Cases

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The authors investigated the distribution of interleukin-2 receptors (TAC antigen) in the lymph nodes of 300 patients with lymphoproliferative disorders. They used fresh-frozen sections to evaluate a possible correlation between the immunophenotype of specific lymphoid disorders and the presence or absence of TAC expression and to determine whether the TAC positivity of lymphoid cells contributes to the characterization of lymphoproliferative processes. All of the cases had previously been studied with a large screening panel of monoclonal antibodies and polyclonal antisera. Among 85 patients with a variety of benign reactive processes, the lymph nodes from 47 contained TAC-bearing lymphocytes in various patterns of distribution. Of 41 patients with Hodgkin's disease, 37 had TAC-bearing lymphocytes. Of 26 B-cell, well-differentiated lymphocytic lymphomas (WDL), 14 were diffusely TAC-positive and one had TAC-bearing cells in random distribution. Six cases of intermediate lymphocytic lymphoma were also studied, and three showed randomly distributed TAC-bearing lymphocytes. Of 19 patients with follicular or follicular and diffuse, poorly differentiated lymphocytic (PDL) lymphoma, 14 were TAC-positive. All 3 diffuse PDL lymphomas studied were TAC-negative. Among 23 cases of B-cell and 5 cases of T-cell mixed cell lymphoma, 15 and three, respectively, had TAC-positive lymphocytes. Of 39 large cell lymphomas (B-cell, 33; T-cell, 6), 14 were TAC-positive. All 13 cases of hairy cell leukemia were diffusely positive. Of 23 T-lymphoblastic lymphomas, only 1 showed positive TAC reactivity, which was focal. Of 5 cases of cutaneous T-cell lymphoma, 2 had TAC-bearing lymphocytes. Our study indicates that the TAC antigen is not lineage-specific, and that it may be expressed by lymphoid cells regardless of their phenotype. (Am J Pathol 1987, 127:27–37)

INTERLEUKIN-2 (IL-2) is an immunoregulatory substance produced by T lymphocytes and is required for human T-lymphocyte proliferation.[1] T lymphocytes also express a specific high-affinity receptor for IL-2 on their cytoplasmic membrane; this receptor is referred to as the TAC antigen.[1] Monoclonal antibodies reactive with IL-2 receptors on lymphocytes have recently been produced which are capable of preventing T-cell proliferation *in vitro* by blocking the IL-2 receptors.[1,2] Recently, it has been shown that IL-2 can also induce B-cell proliferation, and that transformed B lymphocytes can express the TAC antigen.[1,3,4]

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The distribution of the TAC antigen in tissue sections has recently been studied in normal human lymphoid tissue[5] and in Hodgkin's disease[6] and other lymphoproliferative disorders (LPDs).[7] We undertook the present study to 1) investigate the histotopographic localization and pattern of distribution of TAC antigen-positive lymphocytes in a variety of benign and malignant lymphoid disorders, 2) determine whether the presence of TAC-positive cells correlates with immunophenotypes of specific lymphoid disorders, 3) evaluate the relationship, if any, of the expression of the IL-2 receptor by lymphoid cells to a specific stage of lymphocyte differentiation, and 4) determine whether the TAC positivity of lymphoid cells is a marker that has biologic significance.

Materials and Methods

Patient Material

This study is based on the analysis of 281 lymph nodes, 13 spleens, and 6 skin biopsy specimens, involved by a variety of neoplastic and nonneoplastic lymphoid disorders culled from the clinical and research files of the James Irvine Center for the Study of Leukemia and Lymphoma. The samples were evaluated in our laboratory between November 1984 and May 1985. Authorization for the use of the research protocol was granted by the Institutional Review Board of the City of Hope National Medical Center.

Morphologic Studies

Portions of the lymph node tissue sections were fixed in buffered formalin or B5 solution and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin and May Gruenwald-Giemsa for routine histologic examination. Non-Hodgkin's lymphomas (NHLs) were classified according to the Modified Rappaport Classification[8] and the Working Formulation.[9]

Immunohistochemical Studies

Fresh-frozen tissues were prepared for immunohistochemical study according to previously described techniques.[10] Sections of normal tonsil were studied as positive controls by a similar method. Procedures for negative control in each case included the use of irrelevant purified isotopic antibody or the substitution of primary antibody by mouse ascitic fluid or nonimmune serum.

Frozen tissues from all of the specimens were studied with the use of a screening panel of antibodies listed in Table 1. The anti-TAC antibody used in this study was kindly provided by Dr. Thomas Waldmann of the National Cancer Institute. A modification of the avidin–biotin complex (ABC) technique was used for identification of TAC antigens as previously described.[11] Briefly, the cryostat-cut frozen sections were fixed in graded acetone for 5 minutes. The primary antibody was placed on one of two sections at a dilution of 1:50 and allowed to incubate for 30 minutes. After removal of excessive primary antibody by brief washing in modified PBS, sections were overlaid with biotinylated, affinity-purified antimouse antibody at a dilution of 1:100 for 20 minutes. Subsequently, a preformed complex of avidin and biotinylated horseradish peroxidase at a dilution of 1:80 was applied for 15 minutes. After removal of excessive reagent from the tissue surface with an isotonic buffer system, the substrate color reaction product was developed with 3-amino-9-ethylcarbazol (AEC) (Polysciences Inc., Warrington, Pa). Biotinylated, affinity-purified antimouse antibody and avidin–biotin peroxidase complex were obtained from Vector Laboratories, Burlingame, California. To determine coexpression of the TAC and lymphocyte differentiation antigens, we used a double-staining immunohistologic method as previously described.[10] In these cases the color reaction product was produced by both AEC and diaminobenzidine (DAB) (Sigma Co., St. Louis, Mo).

Table 1 — Screening Panel for Frozen Section Immunohistology

Antibody	Primarily detects cellular antigens expressed by	Source
T29/33	Hematopoietic cells	Hybritech, Inc. (San Diego, CA)
B1	B cells	Coulter Immunology (Hialeah, FL)
B2	B cells, dendritic cells	Coulter Immunology
BA-1	Pre-B cells, B cells	Becton Dickinson (Mountain View, CA)
IgM	Pre-B cells, B cells	Tago Laboratories (Burlingame, CA)
IgG	B cells	Tago Labs
IgA	B cells	Tago Labs
IgD	B cells	Tago Labs
κ	B cells	Tago Labs
λ	B cells	Tago Labs
T-11	T cells	Coulter Immunology
Leu-3a	"Helper" T cells, some histiocytes	Becton Dickinson
Leu-2a	"Suppressor" T cells	Becton Dickinson

Evaluation of Immunostained Sections

A section was considered to be TAC-positive when lymphoid cells showed distinctly positive staining of the membrane or membrane and cytoplasm, which

made them easily distinguishable from the adjacent unstained cells or stroma. We used our recently proposed systematic method for the immunohistologic evaluation of LPDs to determine the pattern of distribution of TAC-positive cells in tissue sections.[12] The intensity of immunostaining was graded as markedly positive (+++), moderately positive (++), mildly positive (+), or negative (−). The pattern of immunostaining of individual cells was classified as surface membrane or cytoplasmic, depending upon the predominant localization of the stain. The pattern of distribution of immunostained cells was classified as follicular (F), interfollicular (I), diffuse (D), and combined follicular and diffuse (Y). When the immunostained cells were scattered throughout the sections without a distinct pattern, this was classified as random (R).

Results

Tables 2, 3, 4, and 5 summarize the distribution of the TAC antigen in 281 lymph nodes, 13 spleens, and 6 skin specimens from patients with benign and malignant lymphoid disorders as evaluated on cryostat-cut, fresh-frozen sections. Our series of cases include 85 cases of benign reactive processes, 41 of Hodgkin's disease (HD), 155 of NHL, 13 of hairy cell leukemia (HCL), five of mycosis fungoides, and one of lepromatous leprosy involving skin.

Reactive Processes (Table 2)

Among the 85 cases with a variety of benign reactive processes, 47 (55%) contained TAC-bearing lymphocytes which exhibited surface membrane staining. Although the immunostained lymphocytes in the majority of the sections had a random distribution and lacked a distinct architectural pattern, their localization tended to be most pronounced in the interfollicular areas (Figure 1). In 1 case of reactive follicular hyperplasia, there was a prominent dendritic immunostaining pattern similar to that seen with anti-B2, an antibody reactive with a B-cell–associated antigen and also reactive with dendritic reticulum cells in lymphoid follicles (Figure 2). In addition to lymphocytes, some histiocytes and immunoblasts exhibited relatively strong immunostaining. Unlike the TAC-positive lymphocytes, the histiocytes and immunoblasts had a predominantly cytoplasmic immunostaining pattern. We were unable to identify any TAC-positive plasma cells.

Hodgkin's Disease (Table 2)

Of 41 cases of HD including 6 lymphocyte predominant, 26 nodular sclerosing, four mixed cellularity, two interfollicular, and three unclassified types, 37 (90%) had TAC-bearing lymphocytes which exhibited a surface membrane staining pattern. Like the

Table 2—Distribution of IL-2 Receptor (TAC) in Reactive Lymphoid Disorders, Hodgkin's Disease, and True Histiocytic Lymphoma on Fresh-Frozen Lymph Node Sections

Diagnosis	No. of cases	Immunoreactivity						Positive cases
		Negative	Positive (pattern)					
			F	I	D	Y	R	
Reactive disorders								
Reactive follicular hyperplasia	49	25	1	4	3	4	12	24
Nonspecific lymphoid hyperplasia	17	8	1	2	3		3	9
Necrotizing lymphadenitis	6	0				1	5	6
Granulomatous lymphadenitis	4	1					3	3
Angioimmunoblastic lymphadenopathy	4	1			1		2	3
Pseudolymphoma	3	2				1		1
Dermatopathic lymphadenitis	2	1					1	1
Total	85	38						47
Hodgkin's disease								
Lymphocyte predominance	6	1			3		2	5
Nodular sclerosing	26	2	12		1	6	5	24
Mixed cellularity	4	1			1		2	3
Interfollicular	2					1	1	2
Unclassified	3	0			2		1	3
Total	41	4						37
True histiocytic lymphoma	6	2			2		2	4

F, follicular; I, interfollicular; D, diffuse; Y, follicular and diffuse; R, random.

Table 3—Distribution of Cells Expressing the IL-2 Receptor (TAC) in Human B-Cell Neoplasms on Fresh-Frozen Lymph Node Sections

Diagnosis	No. of cases	Immunoreactivity						Positive cases
		Negative	Positive (pattern)					
			F	I	D	Y	R	
WDL	26	11			14		1	15
Intermediate lymphocytic	6	3					3	3
PDL, nodular	16	4		7		3	2	12
PDL, diffuse	3	3						0
PDL, nodular and diffuse	3	1		2				2
Mixed cell, nodular	10	3		3		2	2	7
Mixed cell, diffuse	5	3					2	2
Mixed cell, nodular and diffuse	8	2		1		3	2	6
Large cell, diffuse	29	22			5		2	7
Large cell, nodular and diffuse	4	1		1			2	3
Undifferentiated	5	4			1			1
Total	115	57						58

F, follicular; I, interfollicular; D, diffuse; Y, follicular and diffuse; R, random.

Table 4—Distribution of IL-2 Receptor (TAC) in Human T-Cell Lymphomas on Fresh-Frozen Lymph Node Sections

Diagnosis	Total no. of cases	Immunoreactivity						Positive cases
		Negative	Positive (pattern)					
			F	I	D	Y	R	
Large cell, diffuse	6	2			4			4
Mixed, diffuse	5	2			3			3
Lymphoblastic	23	22					1	1
Total	34	26						8

F, follicular; I, interfollicular; D, diffuse; Y, follicular and diffuse; R, random.

Table 5—Distribution of IL-2 Receptor in Hairy Cell Leukemia, Mycosis Fungoides, and Lepromatous Leprosy on Fresh-Frozen Spleen and Skin Sections

Diagnosis	Organ	Total no. of cases	Immunoreactivity						Positive cases
			Negative	Positive (pattern)					
				F	I	D	Y	R	
Hairy cell leukemia	spleen	13	0			13			13
Mycosis fungoides	skin	5	3			1		1	2
Lepromatous leprosy	skin	1	0					1	1
Total		19	3						16

F, follicular; I, interfollicular; D, diffuse; Y, follicular and diffuse; R, random.

cases of reactive lymphoid disorders, most of our cases of HD had a random distribution as the predominant pattern. However, a follicular pattern predominated in approximately 50% of the cases of the nodular sclerosis type. In addition to a TAC-positive background lymphoid population, in those cases in which Reed–Sternberg (R-S) cells and their mononuclear variants were confidently identified on frozen sections, they exhibited a predominantly cytoplasmic staining for the TAC antigen (Figure 3).

Non-Hodgkin's Lymphomas

We investigated 155 cases of B-cell, T-cell, and true histiocytic lymphomas (Tables 2–4), and 70 (46%) of them showed positive immunostaining for TAC.

True Histiocytic Lymphoma (Table 2)

The neoplastic cells in 4 of 6 immunologically, cytochemically, and ultrastructurally documented cases of true histiocytic lymphoma showed diffuse (2 cases) or random (2 cases) immunoreactivity with anti-

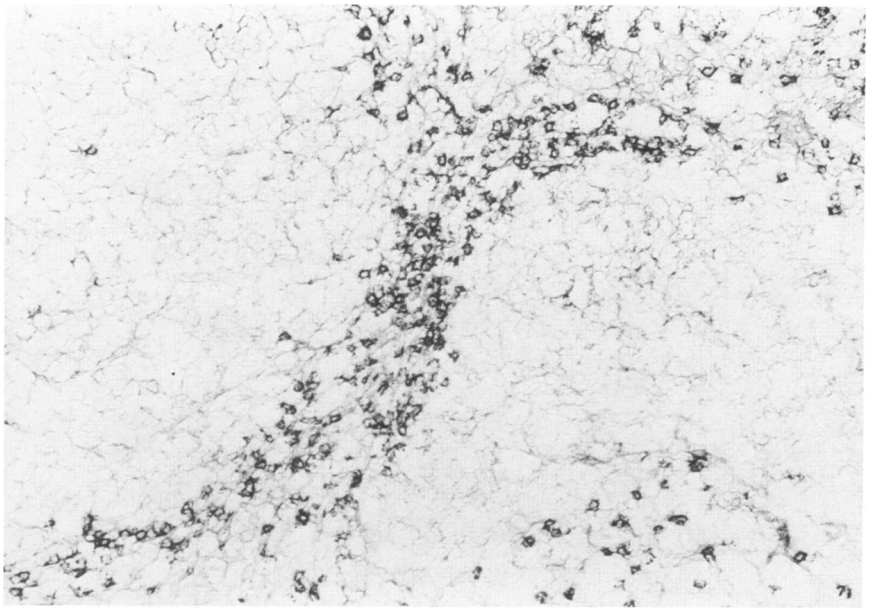

Figure 1—Florid reactive follicular hyperplasia. The TAC-positive lymphocytes are distributed predominantly in the interfollicular areas. (×160)

TAC. The distribution of TAC-positive cells was similar to the pattern of distribution of reactive T cells, but some of the neoplastic cells definitely showed positive staining with anti-TAC antibody.

B-Cell-Derived Lymphomas (Table 3)

Well-Differentiated Lymphocytic (WDL) and Intermediate (Small) Lymphocytic Lymphoma

Fifteen of 26 cases (58%) of WDL were found to be diffusely (14 cases) or randomly (1 case) immunoreactive with anti-TAC antibody. The immunostaining reaction in the majority of the cases was strong, and background staining was insignificant (Figure 4A). Compared with the number of TAC-bearing lymphocytes, the percentage of reactive T lymphocytes, as determined by their reactivity to the T-11 antibody, was extremely low (Figure 4B).

Of 6 cases of intermediate lymphocytic lymphoma, 3 showed positive immunostaining of lymphocytes, which were distributed predominantly in a random pattern.

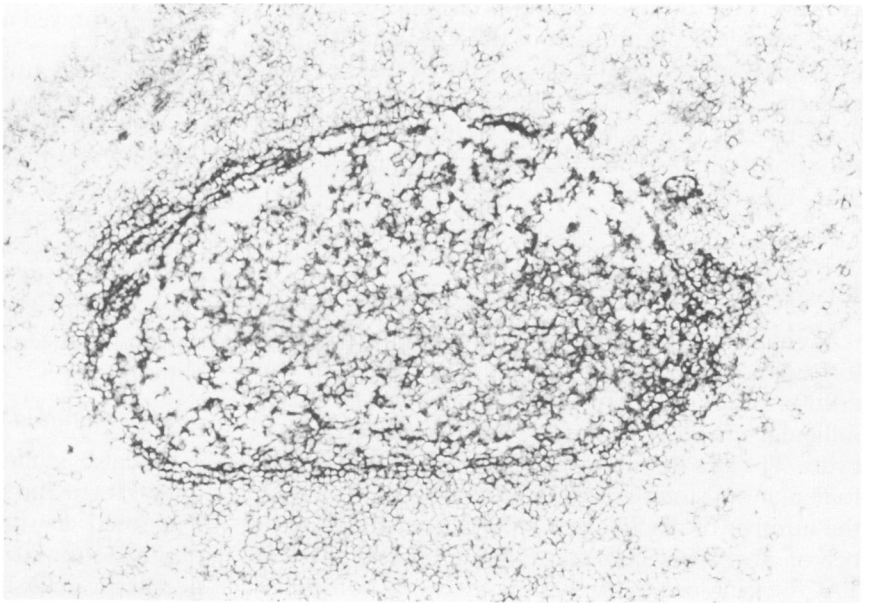

Figure 2—Florid reactive follicular hyperplasia. The TAC-positive lymphocytes are located predominantly in lymphoid follicles in a dendritic pattern. (×160)

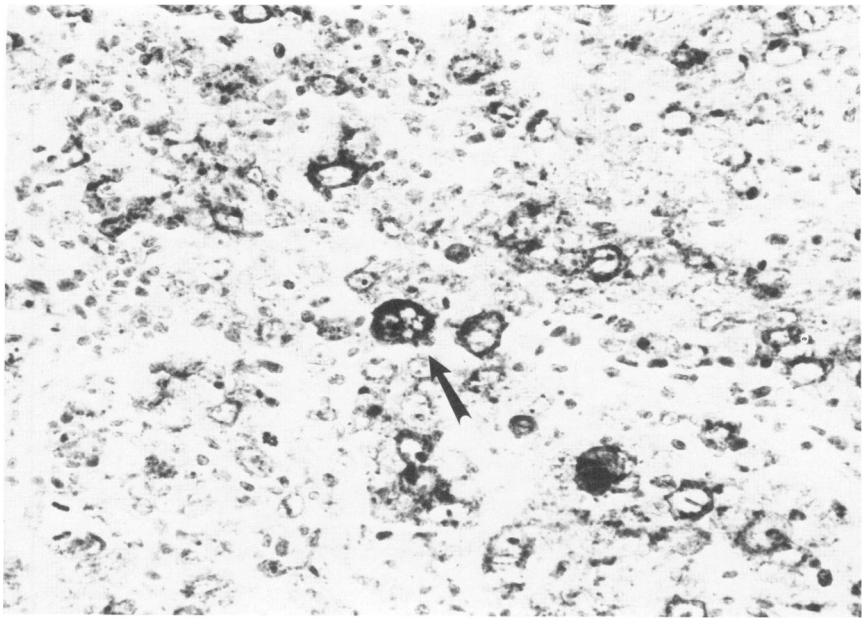

Figure 3—Hodgkin's disease, nodular sclerosing type. The lymphocytes bearing TAC antigen are randomly distributed. The R-S cell (*arrow*) and its mononuclear variants also exhibit a positive reaction in a predominantly cytoplasm staining pattern. (×400)

Poorly Differentiated Lymphocytic (PDL) (Small Cleaved) Lymphoma

In 14 of 22 cases (64%) of PDL lymphoma (16 follicular, 3 follicular and diffuse, 3 diffuse), immunostained lymphocytes were identified. In most of the cases of follicular PDL lymphoma, the distribution of TAC-bearing lymphocytes was predominantly interfollicular, essentially similar to the pattern of distribution of reactive T cells in follicular lymphomas. However, the double-staining procedure done on 2 cases revealed coexpression of the TAC and B1 antigens in neoplastic small cleaved lymphocytes of follicles. In 2 of the 3 cases of follicular and diffuse PDL, TAC-bearing lymphocytes had an interfollicular distribution. All three diffuse PDL lymphomas were TAC-negative.

Mixed Cell Lymphoma

We noted variable positivity in the immunohistologic sections of 23 cases (10 follicular, 8 follicular and diffuse, 5 diffuse) of mixed cell lymphoma. Of the 10 follicular mixed, 7 contained TAC-bearing lymphocytes. The predominant pattern of distribution was follicular and interfollicular. In some cases in which the number of reactive cells approximated the number of neoplastic cells, the precise enumeration of TAC-bearing neoplastic lymphocytes was somewhat difficult. As in follicular PDL lymphoma, double-staining of 2 cases of follicular mixed showed coexpression of TAC and B1 antigens in the neoplastic lymphocytes. The predominant pattern of distribution of TAC-positive cells in the 6 positively staining cases of follicular and diffuse mixed lymphoma was follicular and interfollicular. Of 5 cases of diffuse mixed lymphoma which were proved immunologically to be of B-cell lineage, only 2 had TAC-positive neoplastic and reactive lymphocytes, and in these the staining showed a random distribution.

Large Cell Lymphoma (LCL)

Of 29 cases of diffuse LCL, 5 showed diffuse and two showed randomly distributed TAC-bearing lymphocytes. The immunostaining in both groups of LCL was generally weak and was predominantly located in the surface membrane, although some neoplastic cells with cytoplasmic staining were seen. Of 4 cases of follicular and diffuse LCL, 3 had TAC-bearing lymphocytes in a random or interfollicular distribution.

Undifferentiated (Small Noncleaved) Lymphomas

We also studied 5 undifferentiated lymphomas, one of them Burkitt's and four non-Burkitt's; 1 case, diagnosed as undifferentiated non-Burkitt's lymphoma, showed diffusely distributed TAC-positive neoplastic cells; the remaining cases were negative.

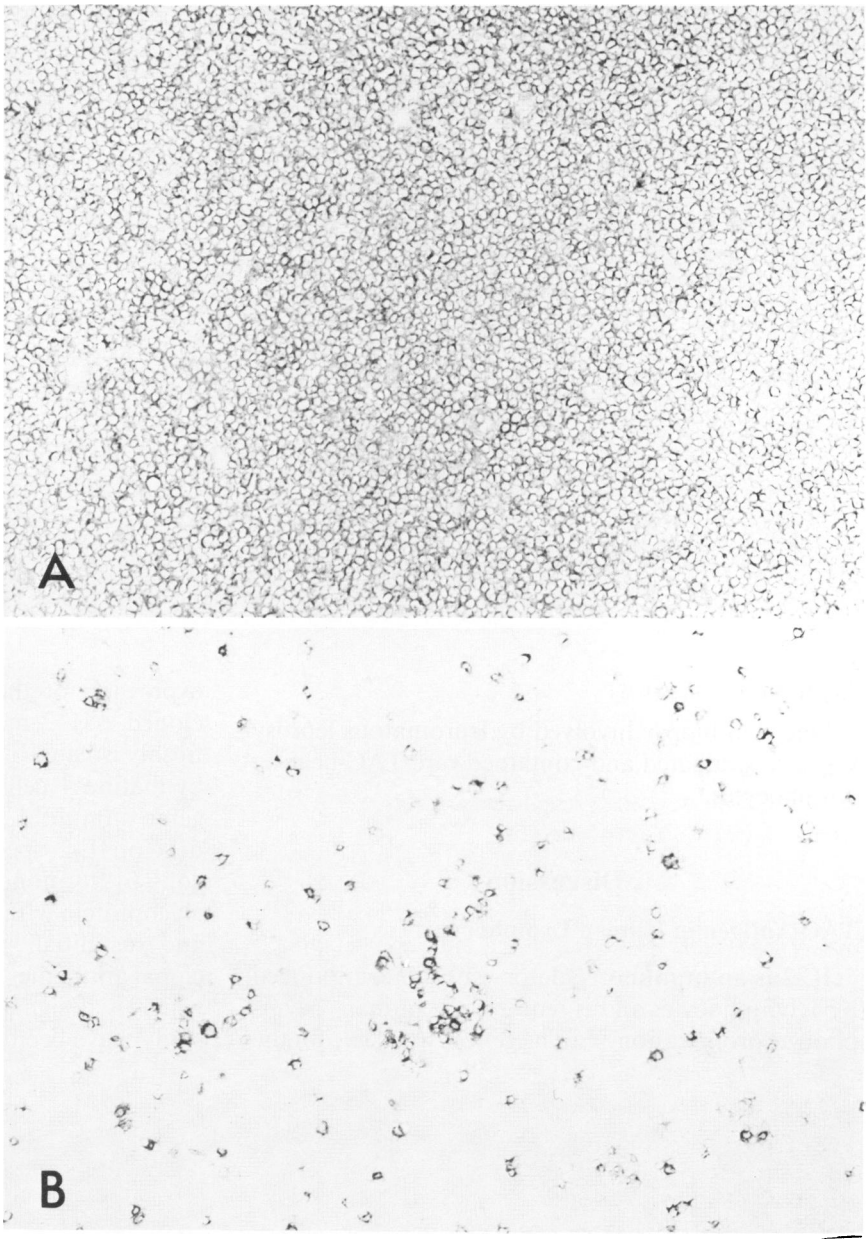

Figure 4A—Diffuse well-differentiated lymphocytic lymphoma of the B-cell phenotype. The neoplastic lymphocytes exhibit surface membrane staining with anti-TAC antibody. (×160) **B**—Diffuse well-differentiated lymphocytic lymphoma (shown in **A**) stained with anti-T-11. Compared with the number of TAC-bearing B lymphocytes (**A**), the percentage of T lymphocytes is very low. (×160)

T-Cell-Derived Lymphomas (Table 4)

Peripheral T-Cell Lymphoma

Neoplastic cells in 4 of 6 cases of LCL were diffusely positive with anti-TAC. Of 5 diffuse mixed cell lymphomas, 3 showed positive immunoreactivity in a diffuse pattern.

Lymphoblastic Lymphoma (LBL)

Twenty-three T-LBLs were studied, and only 1 of these contained randomly distributed TAC-bearing neoplastic lymphocytes.

Hairy Cell Leukemia, Mycosis Fungoides, and Lepromatous Leprosy (Table 5)

Hairy Cell Leukemia

All 13 cases of HCL diffusely stained with anti-TAC (Figure 5).

Mycosis Fungoides

Five cases of mycosis fungoides of the inducer/helper T-cell phenotype were analyzed, and two of these contained TAC-positive cells. In 1 case, their pattern of distribution was diffuse; in the other, the pattern was random (Figure 6).

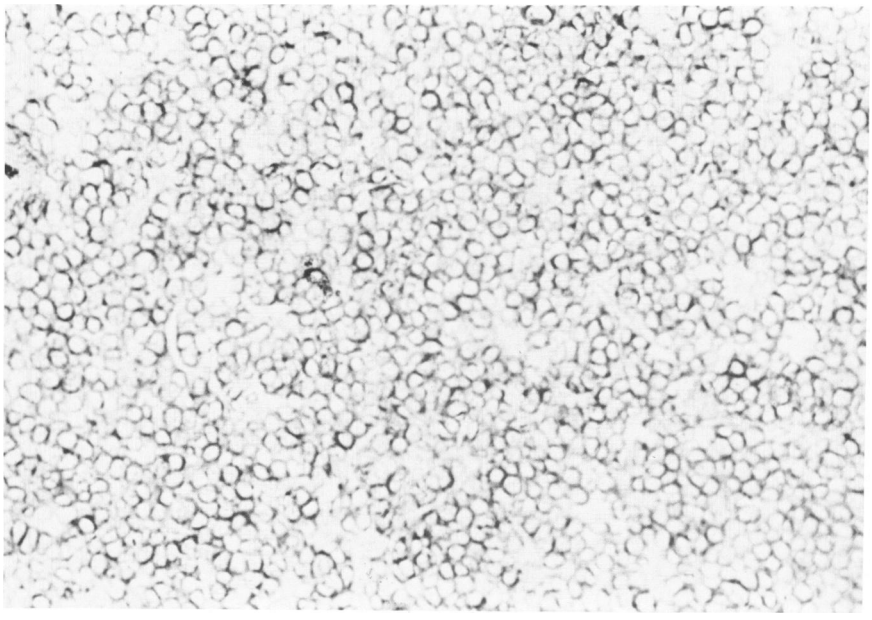

Figure 5—Spleen involved with hairy cell leukemia. The neoplastic cells exhibit strong surface membrane immunoreactivity for the presence of TAC antigen. (×260)

Lepromatous Leprosy

One skin biopsy involved by lepromatous leprosy was also evaluated and contained rare TAC-bearing lymphocytes.

Discussion

TAC Antigen in Human Lymphocytes

IL-2 is an immunoregulatory substance produced by T lymphocytes and is required for human T-lymphocyte proliferation.[1,2,4] The gene responsible for the expression of the IL-2 receptor has recently been cloned.[13] IL-2 may not be present in or released by freshly isolated T lymphocytes, but it can be released by mature T cells that have been activated by mitogenic stimuli.[1,4] Activated T lymphocytes also can develop IL-2–specific membrane-binding sites analogous to hormone receptors. The IL-2 receptor is a glycoprotein with a molecular weight of about 55,000 and was initially defined as the TAC antigen. Monoclonal antibodies directed against this antigen, one of which is designated anti-TAC and was used in this study, have been developed.[1,2] Expression of IL-2 re-

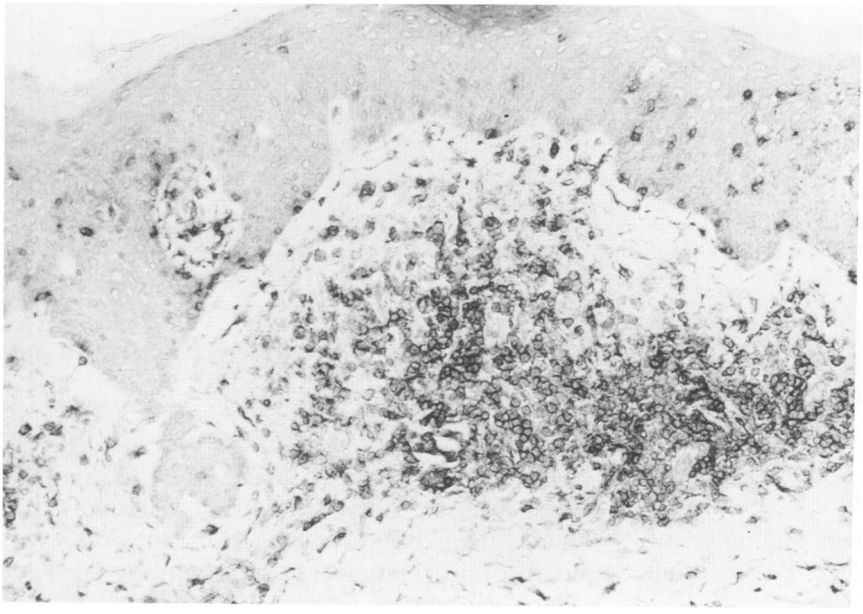

Figure 6—Cutaneous T-cell lymphoma (mycosis fungoides). The neoplastic lymphoid cells show strong surface membrane staining for anti-TAC antibody. (×160)

ceptors is transient and not continuous, but occurs only after appropriate immune stimulation.[14]

Initially, the expression of the TAC antigen was considered to be restricted to the T-cell lineage.[2] More recently, it was shown that activated human B lymphocytes may also express the TAC antigen.[1,3,15] Waldmann et al have demonstrated the expression of the TAC antigen in cloned lines of normal EBV-transformed B lymphocytes and in B-cell lines derived from 5 of 6 patients with HTLV-I associated adult T-cell leukemia.[4] Boyd et al confirmed that activated human B lymphocytes express the TAC antigen and demonstrated that the IL-2 receptors on B lymphocytes are identical to those of T cells.[16] It appears that IL-2, together with other factors produced by T cells, may be a regulator of the differentiation of B cells into immunoglobulin-producing cells and plasma cells.[17,18] In a recent study, Touw et al demonstrated that IL-2 stimulates the neoplastic proliferation of leukemic cells from patients with pre-B acute lymphoblastic leukemia.[19]

IL-2 has also been found to stimulate proliferation of natural killer (NK) cells, presumably through direct action on these cells.[20] Blocking studies with anti-TAC antibodies have demonstrated that IL-2 is necessary for induction of NK cell proliferation.[21] It has been shown that NK cells have receptors for IL-2, which may be structurally similar to the TAC antigen expressed by T and B cells. It is interesting that the single case of our 23 cases of T-lymphoblastic lymphoma included in this study that was TAC-positive also expressed NK-associated antigen.

TAC Antigen in Normal Human Lymphoid Tissues and Reactive Lymphoid Processes

The distribution of TAC-positive cells has recently been studied by Hoffman et al[22] in human fetal lymphoid tissue. Their study showed that the medullary portion of the fetal thymus contains TAC-positive cells. By using a double-staining immunohistologic technique, these investigators demonstrated that TAC-positive cells in human fetal lymphoid tissue express T-cell antigens.[22] Similarily, using anti-TAC antibody, Miyawaki et al were able to identify the cellular localization of TAC-positive cells in human lymph nodes and tonsils.[5] Their study demonstrated that a significant number of TAC-positive lymphocytes were present in these lymphoid organs, with the majority of cells located in T-cell–dependent zones such as the paracortical and interfollicular areas. With a double-staining method, they demonstrated that the TAC-positive cells also express Leu-1, a pan-T-cell antigen. Moreover, the majority of "TAC^{+}-$Leu\text{-}1^{+}$" lymphocytes were of the helper/inducer (Leu-3-positive) subtype; only 20% of TAC-positive lymphocytes expressed the Leu-2 (Cytotoxic/suppressor) antigen.

Our own study of 66 cases of reactive lymphoid and follicular hyperplasia (Table 2) resulted in findings similar to those reported by Miyawaki et al.[5] Of the 66 cases evaluated, 33 (50%) had TAC-bearing lymphocytes, in a quantity and pattern of distribution generally corresponding to those of T cells.

The distribution of TAC-bearing cells was investigated by Modlin et al on fresh-frozen sections from 20 patients with lepromatous and tuberculoid leprosy[23] and equivalent numbers of TAC-positive cells were present in both types of leprosy. We evaluated 4 cases of nonnecrotizing granulomatous lymphadenitis and found randomly distributed TAC-bearing lymphocytes in 3 cases. In addition, we studied a skin biopsy specimen from a patient with lepromatous leprosy and observed rare TAC-positive cells.

TAC Antigen in Hodgkin's Disease

The expression of the TAC antigen was recently studied by Pizzolo et al[6] in 15 cases of HD of various histologic subtypes. These investigators found that, in addition to TAC-positive lymphocytic populations, in majority of the cases, the TAC antigen was expressed by the R-S cells and their mononuclear variants. These findings suggest that some R-S cells and Hodgkin's cells can express the TAC or a structurally similar antigen.

In our 41 cases of HD, the number and pattern of distribution of TAC-positive lymphocytes generally confirmed the findings by Pizzolo et al.[6] In addition to TAC-bearing lymphocytes, most diagnostic R-S cells and their mononuclear varients showed a positive immunoreaction to anti-TAC, which was predominantly cytoplasmic (Figure 3).

TAC Antigen in Human B-Cell Lymphomas

The expression of TAC antigen in HCL,[24] a B-cell neoplasm, as well as in some other B-cell-derived neoplasms,[7] is an additional indication that some B lymphocytes are capable of expressing IL-2 receptors. In a recent study Linder et al evaluated the presence and distribution of the TAC antigen in NHL.[7] Approximately 50% of T-cell lymphomas expressed the TAC antigen. In contrast, neoplastic cells of only 5% of their B-cell-derived lymphomas were TAC-positive.[7] The number of TAC-positive cells was moderately high in LCL and low or absent in small lymphocytic lymphomas.

In contrast to the results reported by Linder et al,

the majority of B-cell–derived lymphomas in our series were TAC-positive. Of 115 immunologically documented B-cell lymphomas, 58 showed focal or diffuse immunoreactivity. In lymphomas with a follicular or follicular and diffuse pattern, the pattern of distribution of TAC-bearing cells generally corresponded to the pattern of distribution of T lymphocytes. Although this finding may indicate that the TAC-positive lymphocytes are reactive T cells, the number of TAC-positive cells in these lymphomas was significantly greater than the number of cells expressing pan-T antigens (Figure 4A and B); this indicates that, in addition to T lymphocytes, some neoplastic and nonneoplastic B lymphocytes also express the TAC antigen. Moreover, double-staining of the selected cases of follicular lymphomas confirmed the coexpression of TAC and B-cell restricted antigens in the neoplastic B lymphocytes. Neither the quantity nor the pattern of distribution of TAC bearing cells helped us to distinguish among various morphologic subtypes of follicular lymphomas (PDL, mixed, large cell) on immunohistologic sections.

The percentage of TAC-positive, diffuse B-cell lymphomas was less than that of follicular and follicular and diffuse B-cell lymphomas. This finding may be correlated to the greater number of reactive T lymphocytes usually present in follicular lymphomas. An interesting observation in our study was the strong expression of the TAC antigen by the neoplastic cells in the majority of our cases of WDL, whereas it was weakly expressed in intermediate lymphocytic lymphoma and absent in diffuse PDL. This finding appears to be correlated with the degree of morphologic differentiation of the neoplastic cells of malignant lymphomas, as proposed by Rappaport in 1957[25]; however, a larger series of cases should be evaluated for confirmation of this preliminary finding. All of our 13 cases of HCL were strongly positive, confirming the previous observations of other investigators regarding the expression of TAC antigen by HCL.[24]

In general, the intensity of the immunostaining reaction of TAC-positive cells in B-cell lymphoma was greater in nonneoplastic than in neoplastic lymphocytes. Careful analysis of the immunostained sections did not reveal any significant correlation between the immunophenotype of the positive cells and the intensity of the immunostaining reaction; B lymphocytes were, in general, as intensely positive as were T lymphocytes.

TAC Antigen in Human T-Cell Lymphomas

Immunoelectron-microscopic localization of the TAC antigen in adult T-cell leukemia/lymphoma was investigated by Shamoto et al,[26] who studied lymph node biopsy specimens from 23 patients. Thirteen patients in their series were HTLV-I-positive, and 10 were HTLV-I-negative. The TAC antigen was identified in all 13 of the HTLV-I-positive and in three of the HTLV-I-negative T-cell lymphomas. Both the plasma membranes and the cisternae of the rough endoplasmic reticulum stained with anti-TAC antibody.

Of our 11 diffuse peripheral T-cell lymphomas (large cell, 6; mixed, 5), 7 (64%) showed diffuse immunostaining of neoplastic lymphoid cells. Of 5 cases of mycosis fungoides, 2 contained diffusely (1) or randomly (1) distributed TAC-positive lymphocytes. A greater percentage of diffuse peripheral T-cell lymphomas than of diffuse B-cell lymphomas were TAC-positive, but the number and distribution of TAC-bearing cells were not helpful in predicting the immunophenotype of either type of diffuse lymphoma. Of 23 T-cell LBL studied with anti-TAC, only one (LBL expressing NK-associated antigens) stained positively. It is possible that the neoplastic cells of T-LBL, which are generally in an early phase of the thymocyte differentiation pathway, may not yet have acquired this particular surface-membrane receptor.

Conclusion

In summary, the results of our study of TAC expression on a variety of well-characterized benign and malignant lymphoid tissues indicate that 1) the presence or absence of the TAC antigen appears to have no significant implications for the immunologic classification of LPD with the exception of HCL, which uniformly expressed TAC antigen; 2) TAC expression by neoplastic cells in LPD occurs in significant numbers of cases; and 3) the TAC antigen is not a lineage-specific marker and may be detected in lymphoid cells regardless of their immunologic phenotype.

Because the IL-2 receptor is a cell surface protein, with dynamic and changing patterns of expression, the heterogeneity of anti-TAC staining in these tissues is not surprising. Variability of anti-TAC staining may be accounted for by 1) the proliferative rate of the neoplastic cells, 2) the proliferative rate of the surrounding or reactive nonneoplastic lymphocytes, and 3) a conformational change of the IL-2 receptor molecule as expressed on the cell surface which "hides" the particular epitope recognized by anti-TAC. Finally, saturation of IL-2 receptors with endogenous IL-2 may account for the lack of TAC staining sites in the tissues we studied.

References

1. Waldmann TA: The structure, function, and expression of interleukin-2 receptors on normal and malignant lymphocytes. Science 1986, 232:727–232
2. Uchiyama T, Nelson DL, Fleisher TA, Waldmann TA: A monoclonal antibody (anti-TAC) reactive with activated and functionally mature human T cells: II. Expression of TAC antigen on activated cytotoxic killer T-cells, suppressor cells, and on one of two types of helper T cells. J Immunol 1981, 126:1398–1403
3. Zubler RH, Lowenthal JW, Erard F, Hashimoto N, Devos R, MacDonald HR: Activated B cells express receptors for and proliferate in response to pure interleukin 2. J Exp Med 1984, 160:1170–1183
4. Waldmann TA, Goldman CK, Robb RJ, Depper JM, Leonard WJ, Sharrow SO, Bongiovanni KF, Korsmeyer SJ, Greene WC: Expression of interleukin 2 receptors on activated human B cells. J Exp Med 1984, 160:1450–1466
5. Miyawaki T, Ohzeki S, Ikuta N, Seki H, Taga K, Taniguchi N: Immunohistologic localization and immune phenotypes of lymphocytes expressing Tac antigen in human lymphoid tissues. J Immunol 1984, 133:2996–3000
6. Pizzolo G, Chilosi M, Semenzato G, Caligaris-Cappio F, Fiore-Donati L, Perona G, Janossy G: Immunohistological analysis of Tac antigen expression in tissues involved by Hodgkin's disease. Br J Cancer 1984, 50:415–417
7. Linder J, Daley DT, Armitage JO, Kay DH, Klassen LW, Weisenburger O: Interleukin-2 receptor (TAC) expression in non-Hodgkin's lymphoma (Abstr). Lab Invest 1985, 52:39A
8. Nathwani BN: The Rappaport classification of the non-Hodgkin's lymphomas: Is it pertinent for the 1980's? Lymphoma II: Controversies in Lymphoma (Including Hodgkin's Disease), Diagnosis and Management. Edited by JM Bennett. Boston: Martinus Nijhoff Publishers, 1983, p 183
9. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphoma: Summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer 1982, 49:2112
10. Sheibani K, Tubbs RR: Enzyme immunohistochemistry: Technical aspects. Sem Diagn Pathol 1984, 4:235–250
11. Hsu SM, Raine L, Fanger H: A comparative study of the PAP method and avidin-biotin-complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981, 75:734–738
12. Sheibani K, Winberg CD, Rappaport H: A systematic approach for immunohistologic classification of lymphoproliferative disorders (Abstr). Lab Invest 1986, 54:58A
13. Leonard WJ, Depper JM, Kanehisa M, Kronke M, Peffer NJ, Svetlik PB, Sullivan M, Greene WC: Structure of the human interleukin-2 receptor gene. Science 1985, 230:633–639
14. Cantrell DA, Smith KA: The Interleukin-2 T-cell system: A new cell growth model. Science 1984, 224:1312–1316
15. Suzuki T, Cooper MD: Comparison of the expression of IL 2 receptors by human T and B cells: Induction of the polyclonal mitogens, phorbol myristate acetate, and anti-μ antibody. J Immunol 1985, 134:3111–3119
16. Boyd AW, Fisher DC, Fox DA, Scholssman SF, Nadler LM: Structural and functional characterization of IL 2 receptors on activated human B cells. J Immunol 1985, 134:2387–2392
17. Kishi H, Inui S, Muraguchi A, Hirani T, Yamamura Y, Kishimoto T: Induction of IgG secretion in a human B cell clone with recombinant IL 2. J Immunol 1985, 134:3104–3107
18. Leibson H, Marrack JP, Kappler J: B-cell helper factors: II. Synergy among three helper factors in the response of T cell- and macrophage-dependent B-cells. J Immunol 1982, 129:1398–1402
19. Tuow I, Delwel R, Bolhuis R, van Zanen G, Lowenberg B: Common and pre-B acute lymphoblastic leukemia cells express interleukin 2 receptors, and interleukin 2 stimulates in vitro colony formation. Blood 1985, 66:556–561
20. Shaw ARE, Bleackley RC, Merryweather JP, Barr PH: Modulation of human natural killer cell activity by recombinant human interleukin 2. Cell Immunol 1985, 90:547–554
21. Schmidt RE, Hercend T, Fox DA, Bensussan A, Bartley G, Daley JF, Schlossman SF, Reinherz EL, Ritz J: The role of interleukin 2 and T11 E rosette antigen in activation and proliferation of human NK clones. J Immunol 1985, 135:672–678
22. Hoffman FM, Modlin RL, Bhoopat L, Taylor CR: Distribution of cells bearing the Tac antigen during ontogeny of human lymphoid tissue. J Immunol 1985, 6:3751–3755
23. Modlin RL, Hofman FM, Horwitz DA, Husmann LA, Gillis S, Taylor CR, Rea TH: *In situ* identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin 2 and its receptor. J Immunol 1984, 132:3085–3090
24. Korsmeyer SJ, Greene WC, Cossman J, Hsu S-M, Jensen JP, Neckers LM, Marshall SL, Bakhshi A, Depper JM, Leonard WJ, Jaffee ES, Waldmann TA: Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukemia. Proc Natl Acad Sci USA 1983, 80:4522
25. Rappaport H: Tumors of the hematopoietic system, Atlas of Tumor Pathology, Section III, Fascicle 8. Washington, DC, Armed Forces Institute of Pathology, 1966, p 97
26. Shamoto M, Suchi T, Uchiyama T: Immunoelectronmicroscopic localization of Tac antigen in adult T-cell leukemia/lymphoma. Am J Pathol 1985, 119:513–516

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